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RESEARCH ARTICLE

Effects of chitosan nanoparticles coating on delaying of seed soybean (*Gycine max*) deterioration

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ABSTRACT

High-quality seed is always demanded by farmers and may result in up to a 30% increase in crop yields. One of the major constraints of soybean cultivation is that soybean seeds quickly deteriorate in storage due to high lipid and protein content. Coating technology with various coating formulas is needed to extend seeds' shelf life. This research aimed to determine the effects of CSNPs (chitosan-wax nanoparticles) and CS (chitosan-wax) as active coating ingredients in delaying the Deterioration of soybean seed quality. Two varieties of soybean seed and three coating treatments (control, CS, and CSNPs) were used during storage for six months. Each treatment was replicated four times, and the observation was conducted monthly. The result showed that coating treatment could significantly suppress the increase in water content and the decrease in protein and fat levels compared to the control. Moreover, the coating also suppresses the respiration rate, ethylene content, and MDA concentration during storage, especially for the Grobogan variety. The coated seed showed a higher vigor index and germination than the controls, particularly in the Dega variety. The CSNPs performed better than CS and control for all the experimental parameters. The germination percentage of the Dega variety soybean seeds after six months of storage at CS and CSNPs coating treatments were 93.33 and 92.67%, respectively, much higher than the Grobogan variety. The initial mositure content much influenced the seed's physical, chemical, and viability during storage.

Keywords: Germination; Shelf life; Storage; Viability

INTRODUCTION

Soybean is one of the most important food crops in the world. Soybean production is being increased to fulfill human consumption, the increasing demand for animal feed, and industrial products. One of the limiting factors for soybean production is the availability of quality seeds (Chirchir et al., 2017; Mohammed et al., 2018). The quality of soybean seeds has rapidly regressed, especially in tropical climates, especially in Indonesia, where temperature variations and relative humidity are quite high. In developing countries, farmers often unwittingly use low-quality seeds obtained from local markets (Sperling et al., 2020). Low-quality seeds can result in low germination and productivity (De Vitis et al., 2020). Seed quality cannot be improved during storage but allows maintaining seed quality until the right period for planting by modifying the storage environment (Zhang et al., 2014; Coradi and Lemes, 2019; Brito et al., 2020).

Modifying the temperature and relative humidity of the storage environment is critical for maintaining quality during storage (Sarath et al., 2016; Bakhtavar et al., 2019; Ebone et al., 2020). Seeds have hygroscopic properties, where changes in the relative humidity of the storage environment can affect the moisture content, shelf life, and germination of seeds (De Vitis et al., 2020). Some studies have reported that soybean seed quality can be maintained for up to three months at a storage room temperature of 25°C if the moisture content of soybean seeds is 12%. Meanwhile, soybean seeds with a moisture content of 15% and stored at a temperature of 15 °C, the quality of soybean seeds can only be maintained for up to 135 days (Zuchi et al., 2013; Cao et al., 2017; Coradi and Lemes, 2018; Wijewardana et al., 2018). However, controlling the temperature and humidity of the seed storage environment requires energy and is costly, and the technology is difficult for seed breeders to implement. One of the technologies that can overcome these problems is seed coating technology.

Seed coating is a mechanism for applying substances to seeds that maintain quality and hasten seed growth (Rocha

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et al., 2019a; 2019b). The application of coating through seed coating increases the germination phase, advances phenological occurrences, improves physio-morphological attributes and yields, and, most important, the efficacy of seed restoration (Javed et al., 2022). The active ingredients that are most commonly reported in coatings include fungicides, insecticides, nematicides, predator deterrents, herbicides, microorganisms, and nutrients (Pedrini et al., 2017; Afzal et al., 2020).

One of the active ingredients for seed coating is chitosan. Chitosan, as an active coating ingredient, is quite effective in maintaining seed quality in soybeans (Zeng et al., 2012), artichoke (Ziani et al., 2010), beans and maize (Godínez-Garrido et al., 2021). Some research shows that chitosan can increase salt tolerance in corn seeds (Guan et al., 2009) and wheat (Hameed et al., 2014; Wang et al., 2016a), and it can improve crop response to biotic and abiotic stress (Falcón-Rodríguez et al., 2007; Wang et al., 2016a). In addition, it increases shelf life and maintains nutritional quality (Sánchez et al., 2015; Wang et al., 2016b; Salvador-Figueroa et al., 2017), controls pests and stimulus growth, and increases productivity in soybean crops (Zeng et al., 2012). However, the application of chitosan as an active ingredient in the coating is still constrained by the difficulty-to-dissolve properties in the water, where chitosan can only dissolve in acidic solutions (Li et al., 2019). Many researchers have developed composite emulsion-based films and coatings. Composite bilayer or emulsion-based coatings and films, combining the properties of hydrophilic biopolymers such as chitosan and the hydrophobic character such as lipid and beeswax, have been developed (Gutiérrez-Pacheco et al., 2020). Application of beeswax and chitosan at 2%, significantly reduced physiological weight loss, disease incidence, maintained firmness and prolonged shelf life of fruits compared with untreated control (Eshetu et al., 2019).

Nanomaterial technology can overcome this problem (Harshil et al., 2016; Maswada et al., 2020). Chitosan nanoparticles are chitosan in nano-size produced through nano- suspension technology. Several studies have reported that the application of chitosan nanoparticles as active ingredients of coatings has been shown to maintain seed quality compared to chitosan applications in chili seeds (Chookhongkha et al., 2013) and wheat (Li et al., 2019). However, there is no scientific literature application of chitosan-wax nanoparticles on soybean seed coatings to maintain quality and increase the shelf life. This study aims to investigate the effects of CSNPs (chitosan-wax nanoparticles) and CS (chitosan-wax) as active coating ingredients in delaying the deterioration of soybean seed quality.

MATERIAL AND METHODS

Research design and plant material

In this study, two soybean varieties, namely Grobogan and Dega, were used. The varieties are obtained from seed producers. Soybean seeds obtained from seed producers are sorted by size to obtain uniform seeds. A total of 500 grams of soybean seeds per experimental unit were coated according to the treatment, and there were three coating treatments control (uncoated), CSNPs (Chitosan-wax nanoparticles) and CS (Chitosan-wax) with four replicates for each treatment. The randomized complete block design for each cultivar was used in this study.

Coating material preparation

Double layers of nano-chittosan and nano-coating were employed to provide seed protection against undesirable environmental conditions. Nano-chitosan was prepared using an ionic gelation technique with sodium tripolyphosphate (Na-TPP). This nanostructured material was used to encapsulate vitamin C which functioned as an antioxidant. Chitosan was dissolved in 1% of glacial acetic acid (Merck) at a concentration of 0.2%. Vitamin C (ascorbic acid, 500 ppm) was added into the chitosan solution and mixed thoroughly. Ionic gelation was carried out with a 0.2% Na-TPP (Sigma Aldrich) by stirring using a magnetic stirrer (Thermo Fisher, Singapore). The formed CSNPs were separated by centrifugation for subsequent use as a coating formula.

Nano-wax preparation was carried out through a nano-emulsification process using an ultra-turrax homogenizer (IKA type T25, Germany). Beeswax 3% was melted, mixed with oleic acid and emulsified into hot water with the addition of an emulsifier of Tween 80 and its combination with triethanolamine (TEA) at the same concentration (5-10%). Emulsification was performed using an ultra-turrax homogenizer at a speed of 11,000 rpm for 10 minutes.

The wax coating without nanostructure was prepared by emulsification of wax mixture using a food blender (Phillips 2115) at the highest speed, while the chitosan coating solution was prepared by dissolving chitosan in glacial acetate acid. Dyes are added to the coating solution to distinguish formulas with and without nanostructures, with red (Allura red food dye E129) and green (Idacol fast green FCF) colors, respectively.

Enrichment ingredients added to the formula were in the form of antioxidants (vitamin C) and biological agents (Rhizobium) to help promote growth. Vitamin C was prepared in an absorbed form inside the nano-chitosan matrix through mixing in an ionic glassing process. Rhizobium is added to the coating system through an encapsulation process in a maltodextrin-based matrix.

The coating material without nanostructure was in the form of bees wax emulsion with a Tween 80 emulsifier and a chitosan solution in glacial acetate with glycerol plasticizer. The beeswax emulsion was prepared using an ultra-turrax homogenizer at the highest speed (12,000 – 15,000 rpm). The composition of the coating formula is presented in Table 1.

Coating application on soybean seed

The coating application was carried out on the seed coater through spraying. The coating was given in the form of multi-layers. In coating applications with a multi-layer technique, the enrichment material was first superimposed on the seed's surface, and then continued with the application of the coating formulation.

Soybean seeds (500 g) were placed into the coating chamber. Firstly, chitosan coating solution (8 mL) was dropped onto the seeds while rotating. Subsequently, talcum powder (8 g) was splashed onto the chitosan-coated seeds followed by wax coating. The seed coater was kept rotating and the compressed air was blown onto the seeds The resulting triple-coated seeds were air dried for 2 mins, then left in the open air for 30 mins before being packaged and stored for 6 months at room temperature.

The observations were made monthly (0, 1, 2, 3, 4, 5, and 6 months) for each treatment on the proximate, respiration, ethylene production, electrical conductivity, malondialdehyde (MDA), vigor, and germination. But the proximate observations are carried out at the storage time's beginning and end.

Proximate analysis

For proximate analysis, the seeds are ground into powder. The sample measured the moisture, total ash, lipid, protein, and carbohydrates according to the Association of Official Analytical Chemists (AOAC, 2002).

Measurement of respiration and ethylene production

The rate of respiration and ethylene production were measured following the method suggested by Arif et al. (2022) with some modifications. The rate of respiration and ethylene production during storage was measured using a Gas Analyzer (Merck Felix, type F-950) by flowing air in the sealed glass bottle into a Gas Analyzer with an airflow rate of 70 ml per minute (Felix instrument). The respiration rate is expressed as ml kg^{-1} h^{-1}, and ethylene production is expressed as µL kg^{-1} h^{-1}.

Table 1: The formula composition of CSNPs and CS coating solution

No	CSNPs coating	CS coating
1	Nano-chitosan: 8 ml	Chitosan: 8 ml
2	Ascorbic acid: 4 mg	Ascorbic acid: 4 mg
3	Talcum: 8 g	Talcum: 8 g
4	Nano wax: 8 ml	Bees wax: 8 ml

The MDA measurement

Malondialdehyde concentration (MDA) analysis was performed following the protocol described by Alsamadany and Ahmeed (2022) with some modifications. The final concentration of MDA was measured using the absorbance at 450, 532, and 600 nm using a UV-2600 spectrophotometer (Agilent, USA). The concentration of MDA was calculated through the formula: $6.45 \times (A532 - A600) - 0.56 \times A450$ (Wu et al., 2022). MDA concentrations are expressed as µmol kg^{-1} based on fresh weight.

Measurement of electrical conductivity

Electrical conductivity is evaluated following the method described by Coradi et al. (2020). The measurements using four lots, each containing 50 seeds per experimental unit, were weighed and placed in an Erlenmeyer with 100 ml of distilled water and kept in an incubator at 25 °C for 24 hours. After incubation, the electrical conductivity of the immersion solution is measured by the digital conductivity meter. Results are expressed in µS cm^{-1} g^{-1}.

Measurement of germination and vigour index

The germination and vigor index observation follows the method described by Coradi et al. (2020). The observations were made by planting soybean seeds on between papers (BP). Four sub-samples consisting of 50 seeds from each experimental unit were placed in rolls of paper moistened with distilled water at 2.5 times the mass of dry paper. Seeds are placed in between two moist germination papers and rolled together to look like a rolled towel. The rolled towels are placed inside the germination cabinet/room for incubation. The seed in the roll paper is placed in a germinator set at 25 ± 2 °C. The evaluation of normal, abnormal, and dead seeds counting was carried out on day-5 and day-8 after planting. The germination and vigour index is expressed in percentages (%).

$$\text{Germination} = \frac{\text{Total number of normal seedling}}{\text{Total number of seed tested}} \times 100\%$$

Statistical analysis

The collected data were analyzed using the analysis of variance. The effect of treatment on parameters was evaluated with the Duncan Multiple Range Test (DMRT) to identify significant differences at a significance level of 5%. All statistical analyses were carried out using SAS Portable 9.13 software.

RESULT AND DISCUSSION

Proximate composition

Seeds that are stored for a certain time will undergo deterioration, including the proximate content. Moisture content is one of the proximate parameters that greatly affect the physiology changing in the seed. In this study, the moisture content in the seeds was maintained with the treatment of CSNPs, especially in the Dega variety, where the moisture content was 6.50% and 7.03% for the beginning and six months after storage, respectively, which showed that it only increased by 0.53% (Table 2). This shows that the moisture content of soybean seeds can be maintained < 14% until the end of storage. The seed moisture content of > 14% can induce oxidative enzyme activity related to reactive oxygen species (ROS), which triggers damage to the tire, while the water content of < 6% causes auto-oxidation due to intensified ROS attacks and cracking in seeds (Silva et al., 2018). For seeds in a dry state, although ROS accumulates, the membrane does not show extended damage, caused by very low mobility and a relatively greater affinity of ROS with other cell fractions, such as proteins and nucleic acids (Bailly et al., 2008). Chitosan has excellent film-forming properties, making it easy to form a semipermeable film on the seed surface that can maintain soybean seeds' humidity and moisture content (Zeng et al., 2012). Therefore, the CSNPs coating treatment is considered to inhibit the increase in moisture content in soybean seeds for up to 6 months.

Apart from the moisture content, lipids are also one of the parameters that affect the viability of the seeds. Lipid peroxidation causes many changes in the membrane that can lead to cell death (Agmon et al., 2018), where changes in composition caused by lipid peroxidation will cause extended membrane damage, will open the pores, and lead to loss of ionic homeostasis, furthermore, the membrane loses its integrity by chemical changes in lipids. In this study, lipid reduction can be inhibited by CSNPs coating treatment, where the lipid content was 13.43% and 12.59% for the beginning and six months after storage, respectively, which means a decrease of 0.84% (Table 2). Data on lipid composition have revealed that lipid degradation slightly decreases the triglyceride content during storage and increases the content of free fatty acids, sterols, and phospholipids, indicating that the lipase in the seeds remains active and alters the integrity of the membrane (Sharma et al., 2007). Therefore, the CSNPs coating treatment is stated to inhibit the decrease in lipid levels in soybean seeds.

In this study, CSNPS coating treatment can inhibit the decrease of soybean seed protein contents during storage, where the protein content at the beginning of storage was 35.38% and 35.27% for the Grobogan and Dega varieties, respectively (Table 2). At six months after storage, protein content decreased by 0.52% and 0.60% for the Grobogan and Dega varieties, respectively (Table 2). The nano-chitosan treatment was also more effective in inhibiting the decrease in protein content for the Dega variety (Table 2). Chitosan can increase the conversion activity of proteases into proteins to inhibit the decrease in protein content in soybean seeds (Zeng et al., 2012). Delaying in decreased protein can maintain protein contents, maintaining cell membrane integrity. Proteins in seeds play a role in maintaining seed viability, where disturbances in protein function in the membrane can interfere with the integrity of the membrane, which is triggered by lipid peroxidation

Table 2: The proximate of soybean seed for different coating treatment

Parameters	Coating treatments	Grobogan variety			Dega variety		
		0 Day	180 Day	Distinction	0 Day	180 Day	Distinction
Moisture content (%)	control	9.32	12.21	2.89[a]	6.50	7.91	1.41[a]
	CS	9.32	12.27	2.95[a]	6.50	7.16	0.66[b]
	CSNPs	9.32	12.39	3.07[a]	6.50	7.03	0.53[b]
Ash (%)	control	3.82	4.26	0.44[a]	5.62	6.02	0.40[a]
	CS	3.82	4.08	0.26[b]	5.62	5.79	0.17[b]
	CSNPs	3.82	3.87	0.05[c]	5.62	5.63	0.01[b]
Lipid (%)	control	12.08	10.55	1.53[a]	13.43	11.61	1.82[a]
	CS	12.08	9.93	2.15[a]	13.43	11.92	1.51[a]
	CSNPs	12.08	10.99	1.09[b]	13.43	12.59	0.84[b]
Protein (%)	control	35.38	33.67	1.71[a]	35.27	33.48	1.79[a]
	CS	35.38	34.63	0.75[b]	35.27	33.98	1.29[a]
	CSNPs	35.38	34.86	0.52[b]	35.27	34.67	0.60[b]
Carbohydrate (%)	control	39.40	39.31	0.09[b]	39.18	41.28	2.10[a]
	CS	39.40	39.09	0.31[b]	39.18	41.15	1.97[a]
	CSNPs	39.40	37.89	1.51[a]	39.18	40.08	0.90[b]

The numbers followed by the same letter in the same column indicate no significant difference by Duncan extension multiple range test (P<0.05)

(Agmon et al., 2018). Likewise, the carbohydrates in soybean seeds are the substrates in forming energy through respiration. During the seed storage process, the carbohydrate content may increase from the decay of cell walls and the synthesis of amino acids into polysaccharides. We predict that the increase in carbohydrate content in the CSNP coating treatment of the Dega variety is caused by the low respiration process.

Respiration and ethylene production during storage

Fresh seeds are living tissue and undergo continuous respiration after post-harvest and storage. Respiration is the process of catabolism or the decomposition of organic compounds into inorganic compounds. The respiration process reconstructs glucose and produces CO_2 as well as energy. Depression of the protective ability to ROS by seeds will cause some physiological imbalances in seeds, such as increased respiration, leading to loss of seed strength and viability (Ebone et al., 2019). With the increase in respiration, ROS production increases, leading to an autocatalytic cycle with lipid peroxidation, and there is an increase in damage to genetic material, which ultimately degrades quality and completely inhibits germination (Ebone et al., 2019).

This study could delay respiration by applying CS and CSNPs coatings in both soybean varieties (Fig. 1). Our study shows that CSNPs positively impact the inhibition of respiration rates compared to CS and controls; this may be due to the nature of CSNPs particles whose surface area is large and small. The results study by Tian et al. (2019) showed that the coating treatment of CSNPs could inhibit the respiration process, increase gas permeability and delay aging in ginkgo Biloba seeds during storage. The coating treatment of CSNPs showed lower CO_2 production during storage (Fig. 1a). This indicated that CSNPs could retard the rate of respiration in the Grobogan variety soybean seeds. The increase in CO_2 production in the fifth month and continued with the decrease in CO_2 in the sixth month shows a decrease in seed viability where many cells have been damaged and dead, so that their respiration has decreased drastically. Meanwhile, CO_2 production in soybean seeds of the Dega variety also increased during the storage period, but in the fourth month, there was a drastic increase in CO_2 production in treatments without coating (control) (Fig. 1b). This indicates that the control treatment has already experienced a significant increase in the seed deterioration.

The respiration rate of soybean seeds of the Grobogan variety tends to be higher than that of the Dega variety. We conjectured that the initial moisture content of the seeds affects the respiration rate of both soybean varieties, where

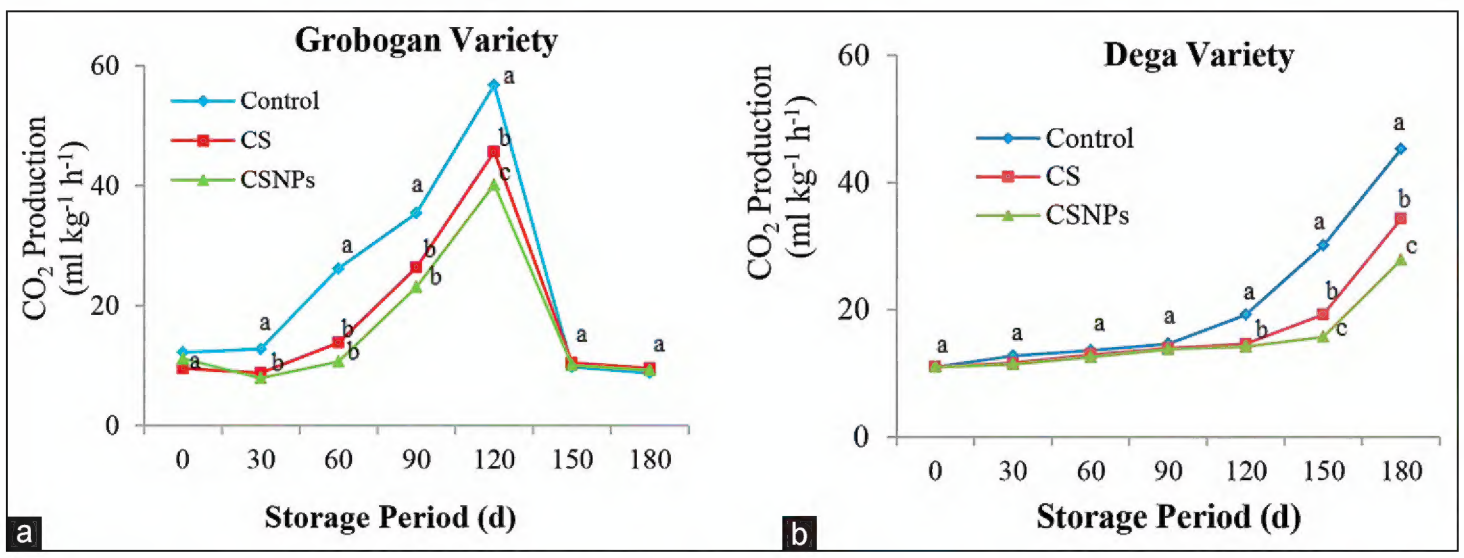

Fig 1. Effect of coating treatments on CO_2 production changes in Grobogan Variety (a) and Dega Variety (b). Data are means of four replicates. Different letters indicate the significant difference in Duncan's multiple range test ($P < 0.05$) on the same day.

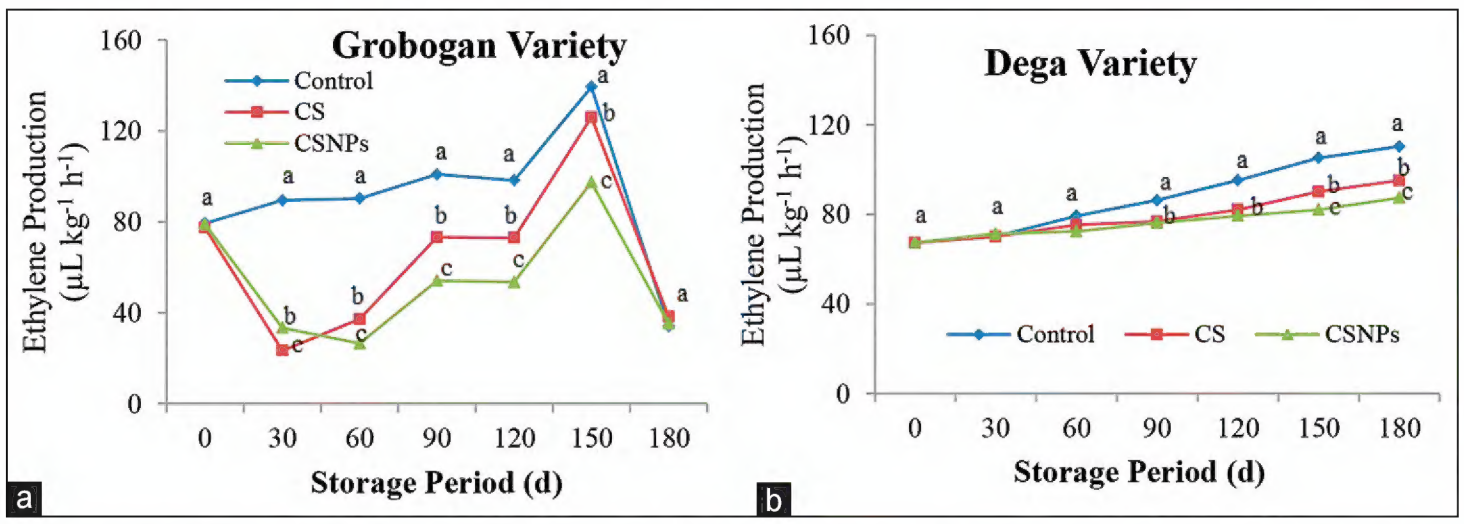

Fig 2. Effect of coating treatments on ethylene production changes in Grobogan Variety (a) and Dega Variety (b). Data are means of four replicates. Different letters indicate the significant difference in Duncan's multiple range test ($P < 0.05$) on the same day.

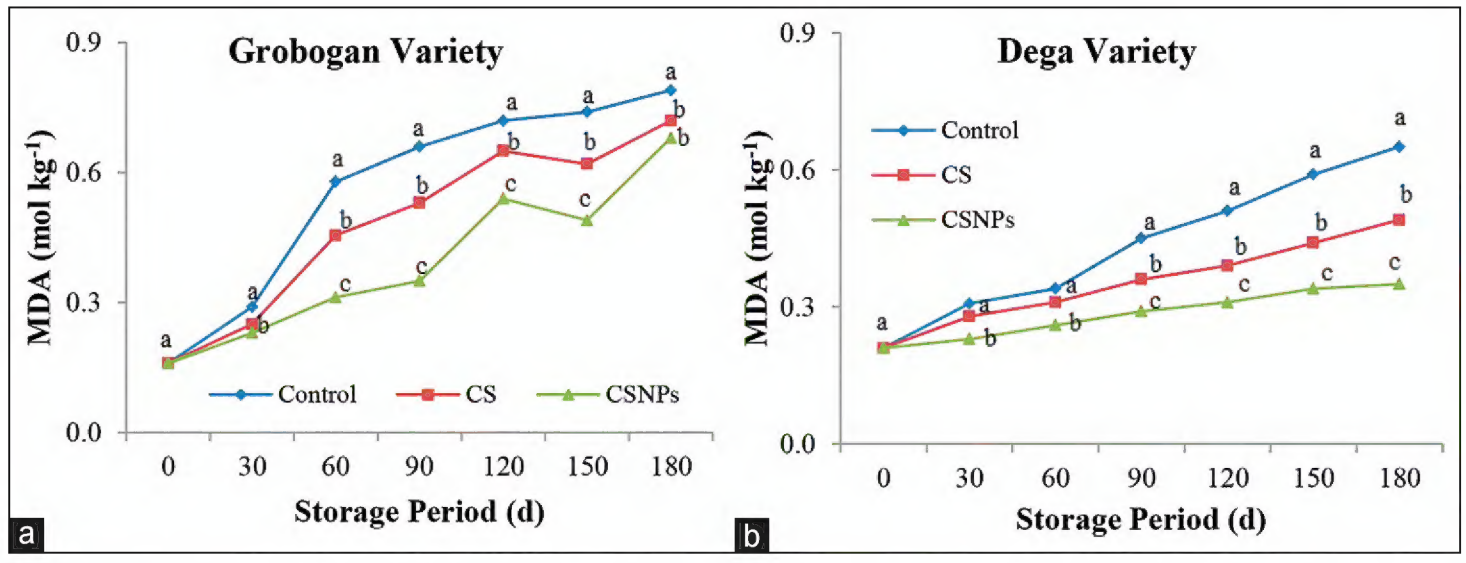

Fig 3. Effect of coating treatments on MDA changes in Grobogan Variety (a) and Dega Variety (b). Data are means of four replicates. Different letters indicate the significant difference in Duncan's multiple range tests ($P < 0.05$) on the same day.

the moisture content of the soybean seeds of Grobogan and Dega varieties is 9.32 and 6.50%, respectively. One of the factors affecting the rate of seed respiration is moisture content (Chidananda et al., 2014). Increasing moisture content by 1% will increase respiration rate (CO_2 production) by 111% in soybean seeds during storage (Ochandio et al., 2017). Hence, the coating treatment of CSNPs is considered appropriate to inhibit the respiration rate of soybean seeds during storage.

Aside from respiration, ethylene production also affects the germination of seeds. The ability to germinate correlates with ethylene production, suggesting that ethylene is involved in the regulation of seed germination and dormancy (Corbineau et al., 2014; Matilla and Matilla-Vazquez, 2008). A decrease in ethylene production can induce seed dormancy; on the contrary, preserving dormancy with various treatments causes an increase in ethylene production (Corbineau et al., 2014). The control treatment showed higher ethylene production in this study than CS and CSNPs coating (Fig. 2). After 3 months of storage, the ethylene production of soybean seeds of Dega variety in the control, CS and CSNPs were 86.32, 76.88, and 76.21 μL kg^{-1} h^{-1}, respectively (Fig. 2b). This indicates that the seeds should be planted immediately because they are not in a state of dormancy (cannot be stored for a long time). Chitosan treatment can inhibit ethylene production in the storage of ginkgo Biloba seeds (Tian et al., 2019). Ethylene production in CSNPs treatment was lower than in CS and control treatment. It can be explained that nanoparticles in coating materials can damage ethylene in a photocatalytic way (De Chiara et al., 2015). Thus, the treatment of CSNPs coatings can inhibit the ethylene production rate in soybean seeds. Furthermore, ethylene production is also related to ROS, whose product can be in the form of MDA. The ROS produced after imbibition affects ethylene production in soybean seeds (Ishibashi et al., 2013).

Malondialdehyde (MDA)

The reduction in protection capacity against ROS in seeds is shown by increased malondialdehyde (MDA) production (Kibinza et al., 2006; Sharma et al., 2013). At the beginning of storage, the MDA levels of soybean seeds in control for the Grobogan and Dega varieties were 0.16 and 0.21 mol kg^{-1}, respectively (Fig.s 3a and 3b). Furthermore, after six months of storage, the MDA levels increased by 5-folds and 3-folds for Grobogan and Dega varieties, respectively (Fig.s 3a and 3b). However, the CSNPs coating treatment showed lower MDA levels than the control and CS-treated (Fig. 3).

Soybean seeds stored under ambient temperature undergo lipid peroxidation, producing a toxic product called MDA (Sharma et al., 2013). The accumulated toxic compounds, respiration, and reduced enzyme activity can lead to the accumulation of toxic compounds that decrease seed viability. According to McDonald (1999), a byproduct of the lipid peroxidation process is malondialdehyde (MDA), which can also destroy proteins and nucleic acids. ROS and MDA trigger severe damage to the genetic material (Shelar et al., 2008). At this stage, disturbances in the mitochondrial membrane increase respiration due to a decrease in energy production of each substrate since there is a reduction in the efficiency of electron transport (Xin et al., 2014). With the increase in respiration, ROS production increases, leading to an autocatalytic cycle with lipid peroxidation, and there is an increase in damage to the genetic material, which ultimately decreases germination. Therefore, the CSNPs coating treatment will effectively inhibit the increase in MDA levels in soybean seeds if the initial moisture content of soybean seeds is low before storage.

Electrical conductivity (EC)

Electrical conductivity is one of the seed vigor tests that indirectly evaluate the degree of cell membrane damage in seeds that have undergone deterioration. The decrease in the physiological potency of seeds is directly related to the increase in the number of ions dissolved in the seed bath due to the destruction of the integrity of the cell membrane (Vieira et al., 2008). The changes in the value of electrical

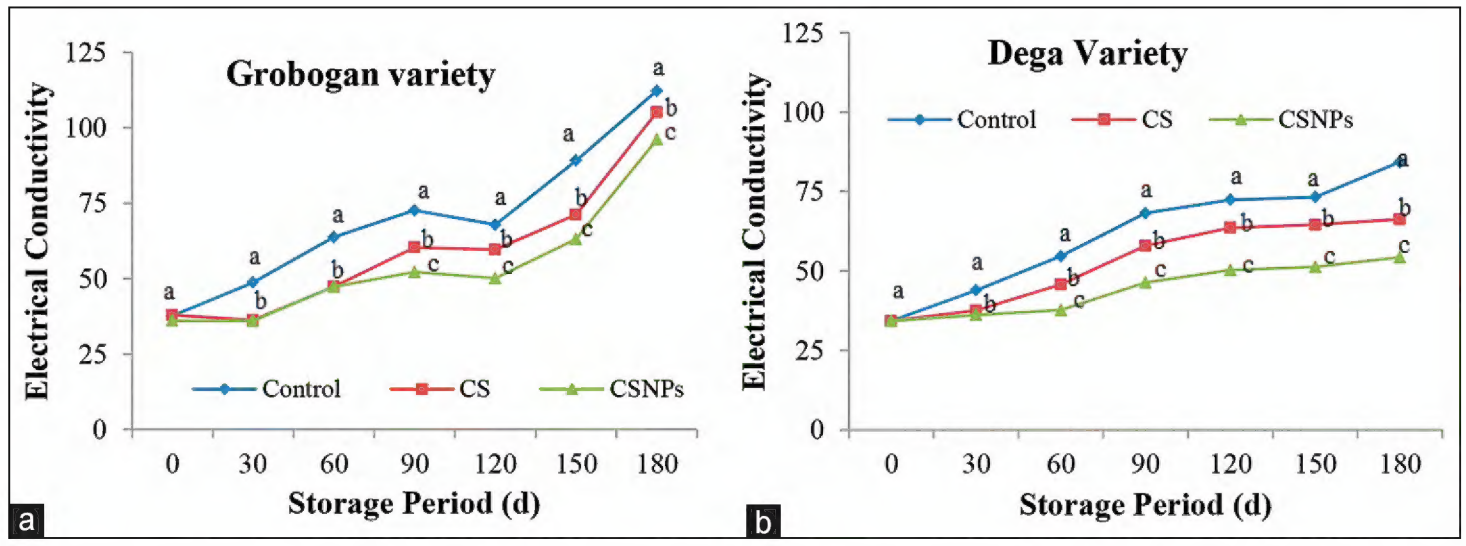

Fig 4. Effect of coating treatments on electrical conductivity changes in Grobogan Variety (a) and Dega Variety (b). Data are means of four replicates. Different letters indicate the significant difference in Duncan's multiple range tests ($P < 0.05$) on the same day.

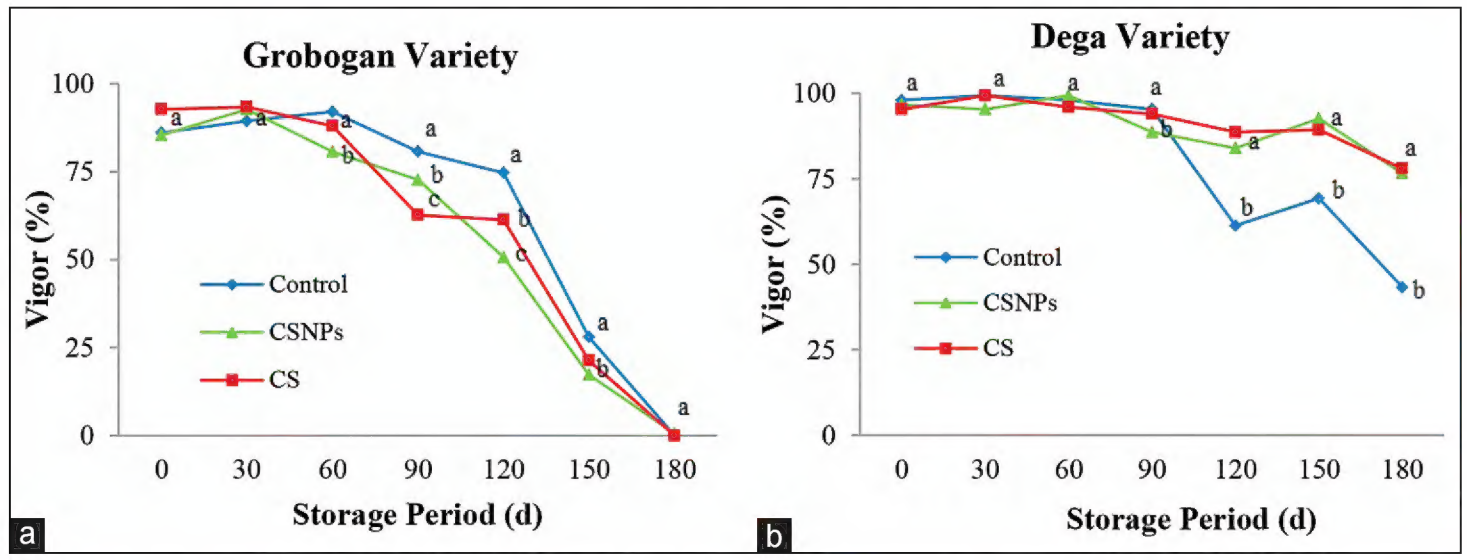

Fig 5. Effect of coating treatments on vigor changes in Grobogan Variety (a) and Dega Variety (b). Data are means of four replicates. Different letters indicate the significant difference in Duncan's multiple range tests ($P < 0.05$) on the same day.

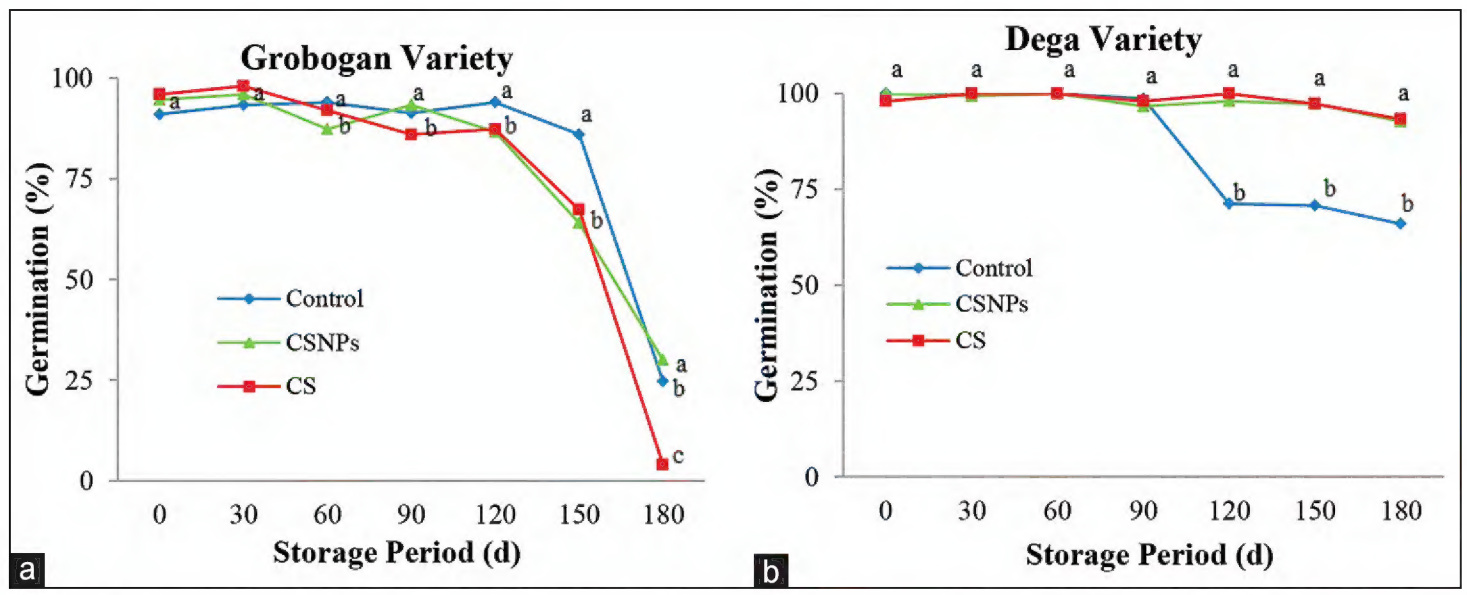

Fig 6. Effect of coating treatments on germination changes in Grobogan Variety (a) and Dega Variety (b). Data are means of four replicates. Different letters indicate the significant difference in Duncan's multiple range tests ($P < 0.05$) on the same day.

conductivity are caused by the changing in the permeability of cell membranes with increased leakage during the shelf life (Wain-Tassi et al., 2012). The value of EC and free fatty acids in seeds will increase during storage (Begum et al., 2013). The lower the number of leaks released in the seed bath solution indicates that the seeds have a high vigor (Carvalho et al., 2009).

However, EC testing to estimate the degree of damage to the membrane will be significant if the soybean seeds tested are stored at a temperature of > 20 ^{0}C (Panobianco and Viera, 2007). The classification of soybean seed vigor levels is based on electrical conductivity values according to criteria by Vieira et al. (2001), namely the low vigor (100-120 $\mu S\ cm^{-1}g^{-1}$), medium (81-100 $\mu S\ cm^{-1}g^{-1}$), and high (<80 $\mu S\ cm^{-1}g^{-1}$). Soybean seeds with a high vigor have an EC value of < 80 $\mu S\ cm^{-1}g^{-1}$ (Neves et al., 2016). Soybean seeds have a higher EC value after storage for 3 months (Carvalho et al., 2016).

In this study, the soybean seeds for both varieties stored at room temperature showed that the EC value in the CSNPs treatment was lower than in the CS and control treatment (Fig. 4). Furthermore, the EC value of the soybean seed of Grobogan variety at six months of storage was 100-120 $\mu S\ cm^{-1}g^{-1}$ in CS and control treatments, while in CSNPS treatment was 80-100 $\mu S\ cm^{-1}g^{-1}$. This shows that the vigor of soybean seeds is classified as the low-medium criterion. In the Dega variety, the EC value of CSNPs and CS coating treatments is classified as a high vigor criterion for up to 6 months of storage. CSNPs treatment can maintain the integrity of cell membranes so that they can inhibit cell membrane leakage. The treatment of CSNPs is considered to inhibit the increase in EC value in soybean seeds during storage.

Vigor index and germination

Vigor index and germination are the indicators that can predict the quality level of the seed. During the storage, soybean seeds will deteriorate, which can be indicated by a decrease in the vigor index and germination. In this study, CS and CSNPs coating treatments showed a higher vigor index and germination compared to the control, especially in the Dega variety (Fig.s 5 and 6). The germination of soybean seeds of Dega variety after 6 months of storage at CS and CSNPs coating treatments was 93.33% and 92.67%, respectively. This suggests that applying chitosan as an active ingredient for coating is effective in maintaining the germination of soybean seeds for up to 6 months. We consider that CSNPs have a larger surface area and a smaller size. The treatment of CSNPs can increase vigor index and germination in wheat seeds (Li et al., 2019), *Zea mays, Brassica rapa* and *Pisum sativum* (Nakasato et al., 2017).

Under uncontrolled conditions, soybean seeds experience a decrease in germination after 90 days of storage and a more significant decrease after 180 days (Lopes et al., 2011; Carvalho et al., 2014). The soybean seeds' germination under uncontrolled environmental conditions (ambient temperature) after 180 and 240 days of storage was 69 and 55%, respectively (Conceiçao et al., 2016). In the Grobogan variety soybean seeds, the vigor index and germination decreased after three months of storage (Fig.s 5 and 6). It is considered that the initial condition of the Grobogan variety is not optimal; this is indicated by the initial water content being relatively high. So, the treatment of CSNPs is considered to maintain the germination of soybean seeds during the storage period if the initial conditions of soybean seeds are of good and optimum quality.

CONCLUSION

The CSNPs-treated delays deterioration in soybean seed until six months storage. The CSNPs-treated suppresses the respiration rate, ethylene content, and MDA concentration during storage. Furthermore, the CSNPs-treated showed a higher vigor index and germination than controls. Seed coating using nano-chitosan is a good alternative to improve seed performance during storage.

AUTHOR'S CONTRIBUTION

Abdullah Bin Arif contributed to the conditioning and assembly of the methodological procedures, to the analysis of the experimental results, and in the writing of the final manuscript. Sri Yuliani contributed to the definition of the methodological aspects in the laboratory, in the analysis and validation of the results. Qanytah contributed to the definition of the methodological aspects in the laboratory, in the analysis and validation of the results. Hernani contributed to the definition of the methodological aspects in the laboratory, in the analysis and validation of the results. Iceu Agustinisari contributed to the definition of the methodological aspects in the laboratory, in the analysis and validation of the results. Christina Winarti was the research leader, supervisor, participated in the conceptualisation and in the writing of the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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